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Response of soil methane uptake to simulated nitrogen deposition and grazing management across three types of steppe in Inner Mongolia, China

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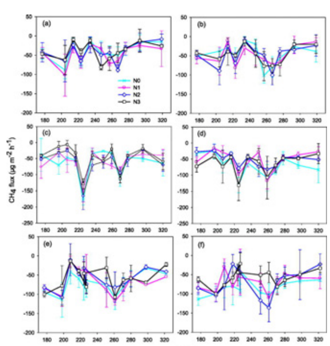
HIGHLIGHTS

- The interaction of nitrogen deposition, steppe types, and fencing management on CH_4 uptake was studied.
- The steppe was a significant sink for CH_4, significantly decreased ($P < 0.05$) with increasing N deposition rates.
- Soil CH_4 uptake was the highest in desert steppe, intermediate in typical steppe, and the lowest in meadow steppe.

GRAPHICAL ABSTRACT



The response of soil methane (CH_4) uptake to increased nitrogen (N) deposition and grazing management was studied in three types of steppe (i.e., meadow steppe, typical steppe, and desert steppe) under grazed and fenced management in Inner Mongolia, China. Results showed that the continental steppe was CH_4 sink (Fig. 2) with the values of 1.12–3.36 kg ha^{-1} over the grass growing season, which was significantly ($P < 0.05$) decreased with increasing N deposition rates. The soil CH_4 uptake rates were highest in the desert steppe, moderate in the typical steppe, and lowest in the meadow steppe. Compared with grazed plots, fencing increased the CH_4 uptake by 4.7–40.2% with a mean value of 20.2% across the three different steppe types. The responses of soil CH_4 uptake to N deposition in the continental steppe varied depending on the N deposition rate, steppe type, and grazing management. A significantly positive correlation between CH_4 uptake and soil temperature was found in this study. Our results may contribute to the improvement of model parameterization for simulating biosphere-atmosphere CH_4 exchange processes and for evaluating the climate change feedback on CH_4 soil uptake.

Fig. 2 Seasonal variation in CH_4 fluxes as affected by increasing N deposition in three different types of continental steppe under grazed and fenced conditions during the growing season from May to October 2012, i.e., meadow steppe grazed (a) and fenced (b), typical steppe grazed (c) and fenced (d), and desert steppe grazed (e) and fenced (f). Error bars indicate standard errors (n = 3) of the mean.

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ABSTRACT

The response of soil methane (CH_4) uptake to increased nitrogen (N) deposition and grazing management was studied in three types of steppe (i.e., meadow steppe, typical steppe, and desert steppe) in Inner Mongolia, China. The experiment was designed with four simulated N deposition rates such as 0, 50, 100, and 200 kg N ha^{-1}, respectively, under grazed and fenced management treatments. Results showed that the investigated steppes were significant sinks for CH_4, with an uptake flux of 1.12–3.36 kg ha^{-1} over the grass growing season and that the magnitude of CH_4 uptake significantly ($P < 0.05$) decreased with increasing N deposition rates. The soil CH_4 uptake rates were highest in the desert steppe, moderate in the typical steppe, and lowest in the meadow steppe. Compared with grazed plots, fencing increased the CH_4 uptake by 4.7–40.2% with a mean value of 20.2% across the three different steppe types. The responses of soil CH_4 uptake to N deposition in the continental steppe varied depending on the N deposition rate, steppe type, and grazing management. A significantly positive correlation between CH_4 uptake and soil temperature was found in this study, whereas no significant relationship between soil moisture and CH_4 uptake occurred. Our results may contribute to the improvement of model parameterization for simulating biosphere-atmosphere CH_4 exchange processes and for evaluating the climate change feedback on CH_4 soil uptake.

1. Introduction

Anthropogenic nitrogen (N) deposition, mainly originating from fertilizer application, fossil fuel combustion, and legume cultivation, has drastically increased around the world since the industrial revolution (Matson et al., 2002; Galloway et al., 2008; Pan et al., 2012). It is thought that this increasing trend will accelerate over the next few decades (Galloway et al., 2004). Elevated N deposition induced by human activities contributes to many negative effects on terrestrial ecosystems, such as reducing biodiversity and causing soil acidification (Liu et al., 2011; Song et al., 2011). In addition, atmospheric N deposition affects a range of biogeochemical processes in terrestrial ecosystems that control the production and consumption of greenhouse gases (GHGs) (Matson et al., 2002; Templer et al., 2012). Recently, the effect of anthropogenic N deposition on GHG fluxes has caused great concern due to the important role that GHGs play in regulating global climate change.

Continental steppe soils are commonly sinks for CH_4 because of their well-aerated mineral soils that support methanotrophic activity, and because the magnitude of the CH_4 sink is affected by steppe type, grazing management, and simulated N depositions rate (Tang et al., 2013; Zhang et al., 2012, 2016). A number of field experiments have been carried out to investigate the individual impact of N deposition (Wei et al., 2014; Zhao et al., 2017), steppe type (Li et al., 2015), and grazing management (Tang et al., 2013; Zhu et al., 2015) on CH_4 efflux in semiarid grasslands. As we know, N deposition was an important factor that controls the potential of steppe soils to act as sinks for atmospheric CH_4 (Ambus and Robertson, 2006; Jassal et al., 2011; Jiang et al., 2010; Li et al., 2012; Mosier et al., 2003; Templer et al., 2012; Chen et al., 2013). The impact of grazing management on CH_4 uptake has been widely investigated in grasslands, showing different impacts of grazing management on CH_4 uptake (Wei et al., 2014; Tang et al., 2013; Zhao et al., 2017). Most studies of soil-atmospheric CH_4 exchange have been conducted in typical steppes in Inner Mongolian steppe (Wang et al., 2005; Liu et al., 2007; Chen et al., 2011a, 2011b, 2013). Across different steppe types, CH_4 uptake in the desert steppe increased 20.4% and 51.2% compared with the typical steppe and meadow steppe, respectively (Tang et al., 2013). However, to the best of our knowledge, few reports are available on soil-atmospheric CH_4 exchanges to study the interaction of steppe types, N deposition, and grazing management. The interactive effect of these three factors on the absorption of CH_4 is not well understood and has not been thoroughly evaluated in Inner Mongolia, China.

Chinese steppes, covering approximately 41.7% of China's land area, are distributed mainly in Inner Mongolia, Xinjiang, Gansu, and the Qinghai-Tibet Plateau (NSBC, 2002). They are part of a continuous expanse of approximately 12.5 million km^2 of temperate grasslands, >8% of the earth's total land surface area (Tang et al., 2013). The aims of this study are to (1) investigate soil-atmosphere CH_4 exchange during the growing season in the three dominant types of steppe ecosystems in Inner Mongolia, China; (2) assess the interactive effects of the N deposition rate, grazing management, and steppe type on the dynamic variation in CH_4 fluxes; and (3) evaluate the relationship between CH_4 uptake and environmental factors.

2. Materials and methods

2.1. Site description

The experiment examined three types of steppe, meadow steppe (120.3 N, 45.1E), typical steppe (116.7 N, 43.6E), and desert steppe (111.9 N, 41.8E), along a 1200-km grassland transect located in Inner Mongolia, China (Fig. 1). The altitudes of these three sites are 656, 1453, and 1428 m, respectively (Cheng et al., 2009). This transect covers a mean annual precipitation (MAP) gradient from 120 to 450 mm and a mean annual temperature gradient from 0.5 to 7.1 °C, and rainfall was the main driving factor of steppe type (Cheng et al., 2009). All three investigated types of steppe were among the dominant steppe types in this region. Experiment site #1 was located on private land rented from local farmers who gave permission to conduct the study at this site. Experiment site #2 was located at a long-term experimental station for the Inner Mongolia grassland ecosystem operated by the Chinese Academy of Sciences. Experiment site #3 was conducted another long-term experimental station for the grassland ecosystem in Siziwang Banner (Fig. 1). None of the field studies involved endangered or protected species.

The meadow steppe is located in the northeastern of Xilingol of Inner Mongolia (120.3 N, 45.1E). The climate is the temperate continental. The mean annual temperature and precipitation is 1.2 °C and 370 mm, respectively, with frost-free period of 106 days (Tang et al., 2013). The annual precipitation mostly occurred during July through August. The soil type is typical kastanozem with soil pH of 6.31 and soil bulk density of 1.06 g cm^{-3}. Soil C and soil N ranged from 0.16–0.27% and 1.46–2.77%, respectively (Table 1). The grassland is dominated by *L. chinensis* (Trin.) Tzvel., *Stipa baicalensis* Roshev., and *Filifolium sibiricum* (L.) Kitam. The ground coverage of vegetation is 60–75% (Tang et al., 2013).

The typical steppe is located in the Xilingol of Inner Mongolia (116.7 N, 43.6E). The climate is the temperate continental and semi-arid. The growing season starts in early May and ends in late September. The annual average temperature is 0.7 °C with a frost-free period of 98 days (Liu et al., 2007). Annual mean precipitation is 330 mm with 60–80% falling between June and August. Soil type is Kastanozem (FAO soil classification), with soil pH of 7.06 and soil bulk density of 1.07 g cm^{-3}. Soil C and soil N ranged from 0.14–0.20% and 1.26–1.52%, respectively (Table 1). The constructive species is *L. chinensis*

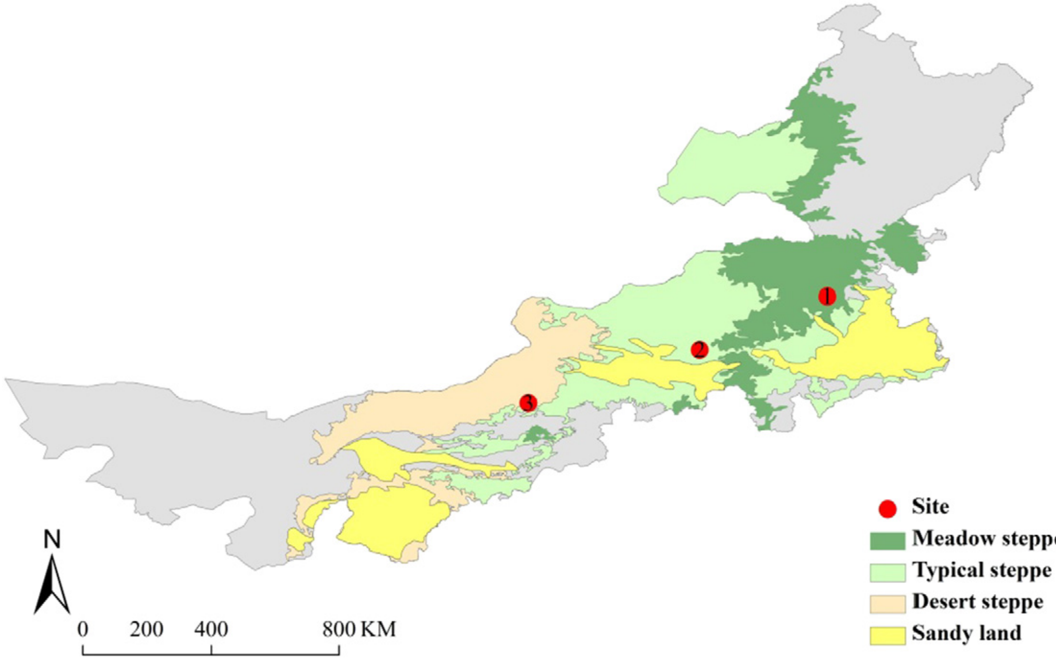

Fig. 1. The locations of the three sampling sites across the different steppe types in Inner Mongolia, China.

and *Stipa grandis*. The ground coverage of vegetation is 50–60% (Tang et al., 2013).

The desert steppe is located in Siziwang Banner in the mid-west of Inner Mongolia (111.9 N, 41.8E). The site has an elevation of 1428 m and is in a temperate continental climate, characterized by a short growing season and long cold winter with a frost-free period of 175 days (Tang et al., 2013). The average annual precipitation is approximately 280 mm, of which nearly 75% falls during June through September. The dominant soil types are Kastanozem (FAO soil classification) or Brown Chernozem (Canadian Soil Classification) with a loamy sand texture Tang et al., 2013) with soil pH 7.65 and soil bulk density 1.25 g cm^{-3}. Soil C and N ranged from 0.12–0.17% and 0.90–1.43%, respectively (Table 1). The grassland is dominated by *Stipa breviflora* Griseb., *Artemisia frigida* Willd., *Cleistogenes songorica* (Roshev.) Ohwi. The ground coverage of vegetation is 18–25% (Tang et al., 2013).

2.2. Experimental treatments

In previous study, based on ten sites observation across urban, suburban, industrial, agricultural, and rural areas during three-year period, the magnitude of total wet and dry deposition of atmospheric N species in Northern China was 60 kg N ha^{-1} with a range from 28.5 to100.4 kg N ha^{-1} because of the high rates of wet deposition and gaseous NH_3 dry deposition (Pan et al., 2012). In China, as the economic development, the N deposition would increase, i.e., inorganic N bulk deposition approximately increased by 25% during the period from 1990s to 2000s (Jia et al., 2014). Based on the research background, in this study, four simulated N deposition rates were selected: N0 (0 kg N ha^{-1}), N1 (50 kg N ha^{-1}), N2 (100 kg N ha^{-1}), and N3 (200 kg N ha^{-1}), respectively. Although the natural N deposition might be < 100 kg N ha^{-1} in our study area, we setup the levels of 100 and 200 kg N ha^{-1} in order to predict the future N deposition rates and the potential effect on CH_4 fluxes in continental steppe in Inner Mongolia, China. The field experiment contained four treatments in triplicates across different steppe types which were arranged randomly.

Urea was applied as a top dressing that was broadcast by hand to simulate N deposition. For the meadow and typical steppes, N deposition was initiated at the onset of the experiment and continued throughout the CH_4 flux observation period. The per-annum amount of added N was divided into four equal portions and applied to the soil at the beginning of each month from June to September in four equal doses except in the typical steppe, where a full dose of N was applied on May 25th, 2012 to maintain the same fertilization management as in previous years. In typical steppe, we setup four treatments using the same fertilization management with the previous year in this long term experiment station in the Xilin River Basin, northern China (Bai et al., 2010; Zhang and Lu, 2017).

At each site, a fenced and grazed steppe was chosen to study the effect of grazing management on CH_4 uptake across the four different N deposition rates. The plots in the meadow and typical steppes were 4 m × 4 m in area, while those in the typical steppe were 8 m × 8 m, all having 2 m wide buffer zones. The dose of N deposition in each treatment was determined by the plot size. A wire fence was constructed at each steppe to protect the study area from livestock.

Table 1
Characteristics of the soil from the three different natural grassland types of meadow steppe, typical steppe and desert steppe, respectively.

Site	Soil depth (cm)	C (%)	N (%)	C/N	Texture (wt%)			Bulk density (g cm^{-3})	Soil pH
					Clay	Silt	Sand		
Meadow steppe	0–10	0.27	2.77	10.14	4.40	50.6	45.0	1.06	6.31
	10–20	0.19	1.74	9.40	5.39	64.1	30.5		
	20–30	0.16	1.46	9.21	6.63	58.9	34.5		
	Mean	0.21	1.99	9.58	5.47	57.9	36.6		
Typical steppe	0–10	0.2	1.52	8.55	3.57	46.4	50.1	1.07	7.06
	10–20	0.16	1.31	7.88	3.55	45.8	50.7		
	20–30	0.14	1.26	8.39	4.16	46.5	49.4		
	Mean	0.17	1.36	8.27	3.76	46.2	50.0		
Desert steppe	0–10	0.17	1.43	8.47	5	42.2	52.8	1.25	7.65
	10–20	0.13	1.1	8.55	5.6	41.5	53.0		
	20–30	0.12	0.9	7.71	5.72	40.3	53.9		
	Mean	0.14	1.14	8.24	5.44	41.3	53.2		

2.3. CH_4 fluxes, soil NH_4^+-N and NO_3^--N, and environmental parameter measurements

The static chamber technique was used to measure CH_4 flux with an interval of three times per month during the grass growing season. The

chamber size was 50 cm × 50 cm × 40 cm. Each base consisted of a white polyvinyl chloride frame with the size of 10 cm in height, 50 cm in inner width, and 50 cm in inner length with a channel on the top. Before sampling, water was irrigated into the channel to form a gastight seal with the 40 cm-tall chamber placed on top of the base. The CH_4 flux was measured at 9:00–11:00 am from 27 May to 16 October 2012. These measurements were conducted on clear days. The gas samples were collected at 0, 10, 20, and 30 min for each measurement using 100-ml polypropylene syringes fitted with three-way nylon stopcocks and then transferred to an air trap (Tedlar, Delin Gas Package Co. Ltd., Dalian, China) for long-term storage. The CH_4 concentration of each sample was analyzed using a gas chromatograph (Hewlett-Packard 5890 Series II, Paco Alto, USA) within a week of collection.

When CH_4 fluxes were monitored, monthly fresh soil samples were collected from the 0–15 cm soil layer at three locations of each plot using a core sampler during the entire growing season from May to October 2012. Fresh soil samples (30 g each) were extracted with 2.0 M KCl (100 mL) in 250 mL plastic bottle on a rotary shaker for 1 h, then the extracts were filtered, and the filtrates were kept in a freezer (4 °C) until analysis. A segment flow analyzer of Skalar (SAN++, the Netherlands) was employed to determine concentrations of NH_4^+-N and NO_3^--N in the soil extracts.

Air temperature (Ta) and soil temperature (Ts) at different depths (0, 3.8, 7.5, and 12 cm) and soil moisture (SM, v/v, %) at 0–3.8, 0–7.5, 0–12, and 0–20 cm were monitored in the soil close to each chamber during gas sample collection. Ta and Ts were measured with a portable digital thermometer (902C, Shengtong Instrument Factory, Hebei, China), and soil moisture (v/v, %) was measured with a portable TDR probe (TDR 300, Spectrum Technologies, Inc., Plainfield, USA). The soil temperature at 20 cm was not measured because it was too difficult to use the portable digital thermometer for the deep soil in the continental steppe.

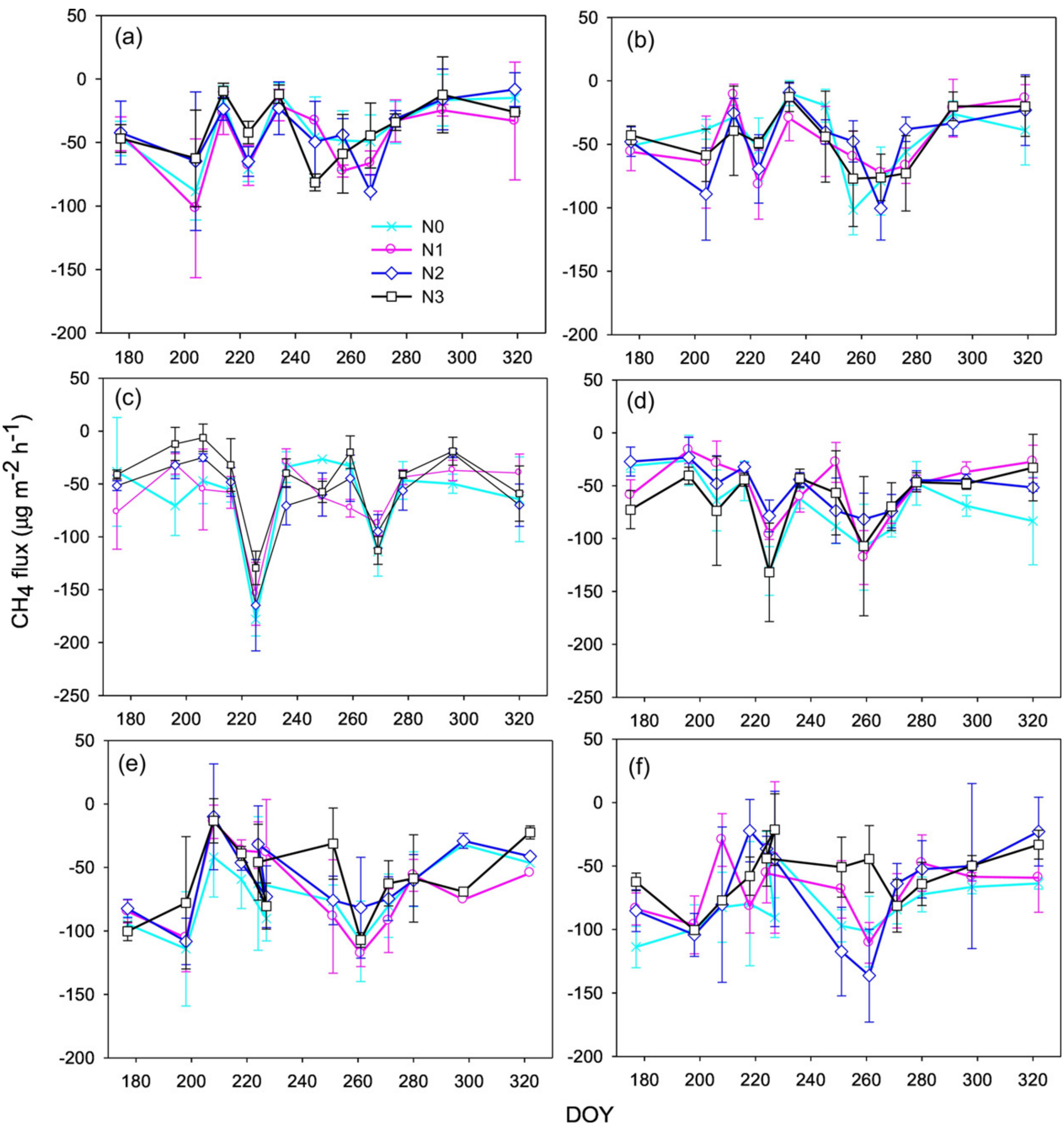

Fig. 2. Seasonal variation in CH_4 fluxes as affected by increasing N deposition in three different types of continental steppe under grazed and fenced conditions during the growing season from May to October 2012, i.e., meadow steppe grazed (a) and fenced (b), typical steppe grazed (c) and fenced (d), and desert steppe grazed (e) and fenced (f). Error bars indicate standard errors (n = 3) of the mean.

2.4. Statistical analyses

The software packages SPSS 16.0 (SPSS, Inc., Chicago, USA) and SigmaPlot 10.0 (Systat Software, Inc., Chicago, USA) were used for statistical data analysis. Repeated-measures analyses of variance (ANOVA) were used to test for differences in CH_4 uptake with nitrogen deposition rate (N), grazing management (G), and steppe type (T) as the main factors. Comparisons of the means were conducted using Tukey's HSD test. Correlation analyses were used to examine the relationships between CH_4 fluxes and the measured environmental variables. The van't Hoff equation ($y = a\exp(bTs)$) was used to calculate the temperature sensitivity ($Q_{10} = \exp.(10b)$) of CH_4 fluxes to changes in Ts. The mean CH_4 fluxes were calculated using a weighted method, and absolute values that indicated the uptake potential that were used during the entire growing season.

3. Results

3.1. Seasonal variation in CH_4 uptake during the entire growing season

Seasonal CH_4 uptake gradually increased from the beginning of the growing season from May to October 2012, reached a maximum and then continuously declined (Fig. 2). Peaks in CH_4 uptake occurred in late June in the meadow steppe, in middle July in the typical steppe, and in middle August in the desert steppe. This indicated that postponed peaks in CH_4 uptake were found in the typical steppe and the desert steppe when compared with the meadow steppe. Both grazed and fenced areas across the continental steppe showed similar patterns in CH_4 uptake, and were CH_4 sinks during the entire measurement period. Relatively lower CH_4 uptake peaks occurred in the fenced typical steppe, indicating that the fenced steppe tends to decrease in CH_4 uptake over the growing season. Generally, the magnitude of soil-atmospheric CH_4 uptake decreased with increasing N deposition rates (Fig. 2).

3.2. Effects of N deposition rate, steppe type, and grazing management on CH_4 uptake

Soil CH_4 uptake tended to decrease with increasing N deposition rates across the three different types of steppe under grazed and fenced conditions (Table 2, Fig. 2). CH_4 uptake at all of the sites with different N deposition rates ranged from 36.1 to 98.0 μg m^{-2} h^{-1} with a mean of 54.7 μg m^{-2} h^{-1} over the entire growing season. The measured mean CH_4 uptake for the N0, the N1, the N2, and the N3 treatments was 65.2, 57.6, 51.3, and 44.7 μg m^{-2} h^{-1}, respectively, whereas the median values of CH_4 uptake were 65.3, 57.5, 50.5, and 44.2 μg m^{-2} h^{-1}, respectively (Fig. 3a). Compared with the control treatment, N deposition decreased the seasonal CH_4 uptake by 12.0–32.4% across the three different steppe types (Table 2). These findings implied that with the increasing N deposition, the sink potential of atmospheric CH_4 would decrease in continental steppes.

Soil CH_4 uptake during the growing season was significantly ($P <$ 0.001) affected by the different types of steppe in the following order:

Table 2
Comparison of the mean and cumulative CH_4 uptake in the three types of grassland under different N deposition rates and grazing conditions. Values within the same column within different steppe types with the same letters (A, B, C, and/or D) are not significantly different at the 0.05 level.

Site	Treatment	Grazed		Fenced	
		Mean CH_4 uptake (μg m^{-2} h^{-1})	Total CH_4 uptake (kg ha^{-1})	Mean CH_4 uptake (μg m^{-2} h^{-1})	Total CH_4 uptake (kg ha^{-1})
Meadow steppe	N0	42.7A	1.47	47.2A	1.62
	N1	40.3A	1.38	45.9A	1.58
	N2	36.1AB	1.24	44.2AB	1.52
	N3	32.6B	1.12	40.7B	1.40
	Mean	**37.9**	**1.30**	**44.5**	**1.53**
Typical steppe	N0	60.7A	2.08	72.9A	2.33
	N1	57.6A	1.98	60.3B	2.04
	N2	50.0AB	1.72	54.9C	1.75
	N3	43.6B	1.50	45.3D	1.64
	Mean	**53.0**	**1.82**	**58.4**	**1.94**
Desert steppe	N0	69.9A	2.40	98.0A	3.36
	N1	60.8AB	2.09	80.5B	2.76
	N2	52.4B	1.80	70.4C	2.42
	N3	46.9C	1.61	58.9D	2.02
	Mean	**57.5**	**1.98**	**77.0**	**2.64**

The averaged values of four treatments across different steppe types were shown in bold texts.

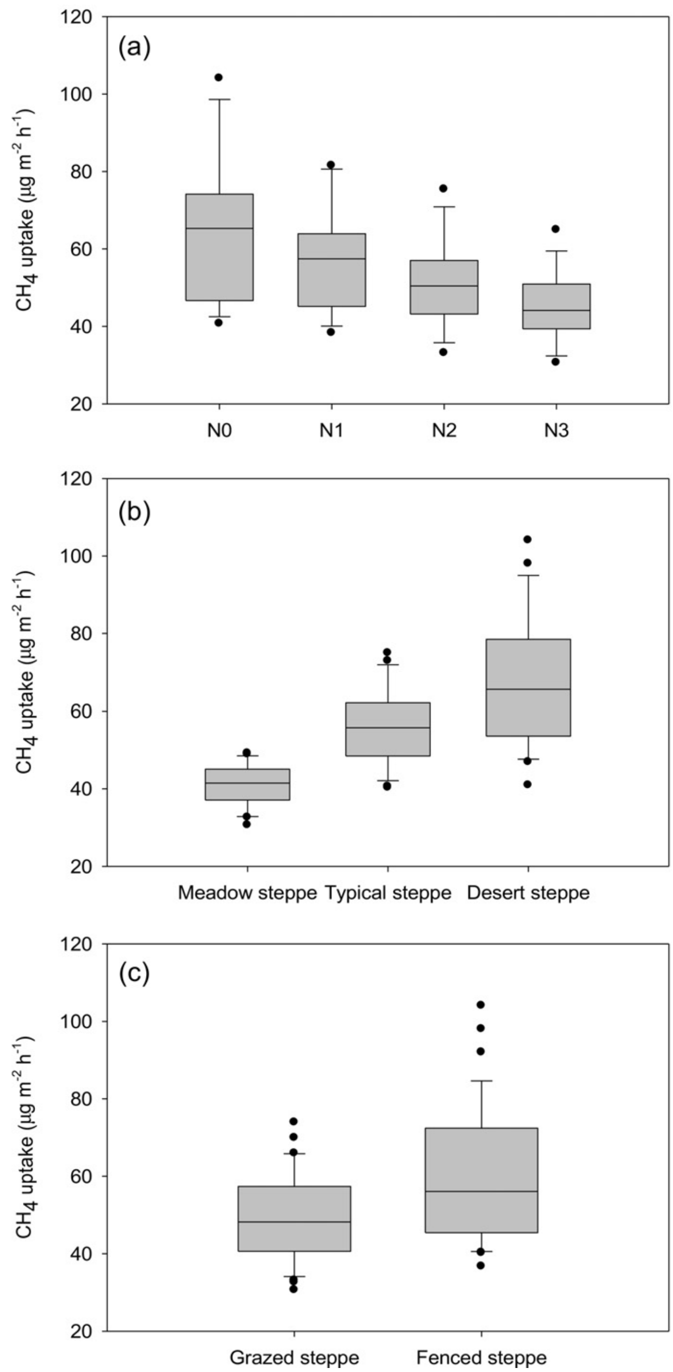

Fig. 3. Mean CH_4 uptake as affected by the N deposition rate (a), types of steppe (b), and grazing management (c) in the continental steppe during the growing season. Additional values are represented by boxplots representing the median (line in the box), mean (square), upper and lower quartiles (vertical line), and minimum and maximum or outliers (points).

desert steppe > typical steppe > meadow steppe (Fig. 3b; Table 3). Soil CH_4 uptake rates in the three types of steppe varied markedly, from 41.2 to 67.2 μg m^{-2} h^{-1}, with a mean value of 54.7 μg m^{-2} h^{-1} during the growing season. Soil CH_4 uptake in the desert steppe showed the greatest CH_4 uptake rate of the three steppe ecosystems with a mean value of 57.5 μg m^{-2} h^{-1} in grazed steppe, which was 51.6% and 8.54% larger than in the typical and meadow steppes, respectively; with a mean value of 77.0 μg m^{-2} h^{-1} in fenced steppe, which was 72.9% and 31.9% higher than in the typical and meadow steppes, respectively. These findings suggest that the global warming potential of continental steppe ecosystems could be overestimated or underestimated if only data from an individual steppe type are considered.

Soil CH_4 uptake during the growing season was also affected ($P < 0.001$) by grazing management in the three types of steppe (Fig. 3c; Table 3). The mean CH_4 uptake was 49.5 and 59.9 μg m^{-2} h^{-1} under grazed and fenced steppe, and the median values of CH_4 uptake were 48.2 and 56.1 μg m^{-2} h^{-1}, respectively. Compared with the grazed sites, fenced steppes increased the CH_4 uptake by 4.7–40.2% with a mean value of 20.2% across the three different steppe types (Fig. 3c; Table 2).

The repeated-measures ANOVA analysis indicated that the N deposition rate, grazing management, and steppe type significantly affected CH_4 uptake over the growing season but that there was no interactive effect of these three factors (Table 3). An interaction between grazing and steppe type on CH_4 uptake was found in this study ($P = 0.047$; Table 3). In the typical and desert steppes, both nitrogen deposition and grazing management were important factors controlling CH_4 uptake ($P = 0.001$–0.131; Table 3).

3.3. Relationship between CH_4 uptake and soil parameters

Soil CH_4 uptake in the three different types of steppe was positively correlated with the soil temperature at different depths at each site (Table 4). The soil temperature of the surface layer played a much more substantial role in controlling CH_4 uptake in grazed steppes, i.e., changes in the soil temperature of the surface layer could explain 76% and 45% of the variation in CH_4 uptake in the grazed typical steppe and desert steppes, respectively. However, in the fenced steppes, the soil temperature at deeper soil depths (12 cm) was the main controlling factor of CH_4 uptake. No significant correlation between CH_4 uptake and soil temperature was found in the meadow steppe because complex soil and vegetation conditions occurred at this site, and soil temperature may not be the main factor controlling CH_4 uptake.

The Q_{10} of CH_4 oxidation varied between 2.08 and 2.48 and between 1.27 and 1.63 in the typical steppe under grazed and fenced conditions, respectively, which were substantially larger values than those in the meadow and the desert steppes (Table 4). There was no significant correlation between CH_4 uptake and simultaneously measured soil moisture in the grassland transect over the growing season (data not shown). This comparison indicated that soil CH_4 consumption in the typical steppe might be more sensitive to changes in global temperature than in the meadow and the desert steppe regions in Inner Mongolia; soil moisture was not the most important factor controlling CH_4 uptake in areas where there might be much more important factors controlling CH_4 uptake, such as soil nutrition, during the measurement period.

Table 3
Repeated-measures ANOVA of CH_4 uptake during the growing season with N deposition rate (N), steppe type (T), and grazing management (G) as the main factors.

	Model	Mean square	F values	*P*
Meadow steppe	G	297	2.06	0.170
	N	94	0.66	0.590
	N*G	9	0.06	0.980
Typical steppe	G	160	2.535	0.131
	N	531	8.426	0.001
	N*G	34	0.545	0.659
Desert steppe	G	2346	16.6	0.001
	N	1115	7.89	0.002
	N*G	71	0.50	0.687
Three types of steppe	N	1445	12.45	0.000
	G	2044	17.61	0.000
	T	4069	35.06	0.000
	N*G	43	0.37	0.772
	N*T	148	1.27	0.287
	G*T	379	3.27	0.047
	N*G*T	35	0.31	0.931

Table 4
Parameters of the regressions between CH_4 flux and soil temperature at different depths (0, 3.8, 7.5, and 12 cm) in the different steppe types under grazed and fenced conditions during the growing season. Q_{10} represents the exponential change in CH_4 flux resulting from a change in temperature by 10 °C. Q_{10} of meadow steppe under grazed and fenced conditions was not presented since there was no significant correlation between CH_4 uptake and soil temperature ($P = 0.076$–1).

Site	Steppe management	Soil depth (cm)	$F = ae^{bx}$				
			a	b	R square	P	Q_{10}
Meadow steppe	Grazed	0	23.09	0.022	0.28	0.076	–
		3.8	24.60	0.020	0.24	0.140	–
		7.5	23.52	0.024	0.25	0.120	–
		12	21.69	0.029	0.27	0.090	–
	Fenced	0	44.65	0.002	0.0037	0.820	–
		3.8	46.42	0.0002	0.0039	0.980	–
		7.5	46.65	0.0002	0	1	–
		12	46.65	0.0003	0	1	–
Typical steppe	Grazed	0	6.88	0.076	0.76	<0.0001	2.14
		3.8	9.63	0.082	0.59	<0.0001	2.27
		7.5	10.52	0.091	0.52	<0.0001	2.48
		12	17.85	0.073	0.35	0.020	2.08
	Fenced	0	33.27	0.024	0.33	0.028	1.27
		3.8	27.86	0.041	0.35	0.019	1.51
		7.5	28.46	0.046	0.38	0.011	1.58
		12	28.65	0.049	0.40	0.0078	1.63
Desert steppe	Grazed	0	32.71	0.027	0.45	0.0024	1.31
		3.8	31.24	0.030	0.41	0.0053	1.35
		7.5	30.05	0.034	0.43	0.0039	1.40
		12	31.39	0.034	0.39	0.0086	1.40
	Fenced	0	49.20	0.014	0.25	0.110	1.15
		3.8	46.38	0.017	0.27	0.080	1.19
		7.5	45.75	0.019	0.27	0.070	1.21
		12	42.99	0.023	0.31	0.040	1.26

4. Discussion

4.1. Effect of simulated N deposition on CH_4 uptake

The annual cumulative CH_4 uptake in the continental steppe sites varied from 1.12 kg ha^{-1} to 3.36 kg ha^{-1} (Table 2), which is comparable to the range of CH_4 uptake in temperate grasslands of Colorado (Mosier et al., 1991), in typical grasslands in Inner Mongolia (Wang et al., 2005; Zhang et al., 2016), in alpine meadows on the Qinghai-Tibetan Plateau (Jiang et al., 2010; Kato et al., 2011; Zhao et al., 2017; Li et al., 2015; Zhu et al., 2015; Wei et al., 2014), and in the degraded steppes of Inner Mongolia (Chen et al., 2013). The repeated-measures ANOVA test showed that CH_4 uptake rates in the N treatment plots in the three different types of steppe significantly decreased with increasing N deposition rates (Table 3). It was reported that the N deposition rate has increased in Inner Mongolia (Chen et al., 2013; Galloway et al., 2003, 2004). Therefore, it is reasonable to assume that increased N deposition induced by anthropogenic reactive N emissions will most likely reduce CH_4 uptake in the continental steppe in Inner Mongolia, China.

Nitrogen addition may increase (Saari et al., 2004; Veldkamp et al., 2001), decrease (Domingues et al., 2007; Liebig et al., 2008; Mosier et al., 1996) or have no effect on (Phillips and Podrebarac, 2009; Sawamoto et al., 2010; Van den Pol-van Dasselaar et al., 1999) CH_4 uptake in grassland ecosystems, appearing to depend on the form and rate of N deposition and soil properties (Rigler and Zechmeister-Boltenstern,

1999). In our study, both under grazed and fenced steppe types, soil CH_4 uptake tended to be decreasing with the increasing simulated N deposition (Table 2). Across three steppe types, ANOVA analysis indicated N deposition was of great importance in impacting CH_4 uptake in Inner Mongolia, China (Table 3, $P = 0.000$). This might be partially attributed to the increasing NO_3^--N content in soils across different steppe types (Fig. 4). Across the steppe transact in Inner Mongolia, relatively higher NO_3^--N content was found in soils which determined the monthly variations of total N concentration of NH_4^+-N and NO_3^--N, especially under grazed steppe. Accordingly, relatively lower CH_4 uptake occurred under grazed steppe (Fig. 4; Table 2).

Serious debates have focused on the role of NH_4^+-N and NO_3^--N on soil CH_4 consumption; some studies have demonstrated that increased soil NH_4^+-N may significantly reduce CH_4 oxidation rates (Bodelier and Laanbroek, 2004). Previous studies also have shown that CH_4 oxidation in grassland soils can be inhibited by the addition of NH_4^+-N fertilizers via affecting CH_4 monooxygenase of methanotrophs (Carlsen et al., 1991; Schnell and King, 1994; Bodelier and Laanbroek, 2004; Saari et al., 2004; Dittert et al., 2005; Jacinthe and Lal, 2006). However, several studies have also reported that NO_3^--N rather than NH_4^+-N had the greatest inhibitory effect on CH_4 oxidation in soils (Reay and Nedwell, 2004; Wang and Ineson, 2003; Xu and Inubushi, 2004; Xu and Inubushi, 2007). The mechanism of NH_4^+-N and NO_3^--N on CH_4 uptake in continental steppe needs further field observations and lab incubation experiment in the future.

4.2. Effect of steppe types on CH_4 uptake

The soil CH_4 uptake rates (36.1 to 98.0 μg m^{-2} h^{-1}) presented in this study fell within the range of those observed in other arid or semi-arid ecosystems (2–105 μg m^{-2} h^{-1}), such as prairies (Mosier et al., 1996), and steppes (Wang et al., 2005; Chen et al., 2011a; Liu et al., 2008, 2009). The difference in the CH_4 uptake rates in the different types of steppe may be attributed to differences in soil characteristics and soil water content (Luo et al., 2013). The soil pH and soil bulk density increased from the meadow steppe in the eastern region to the

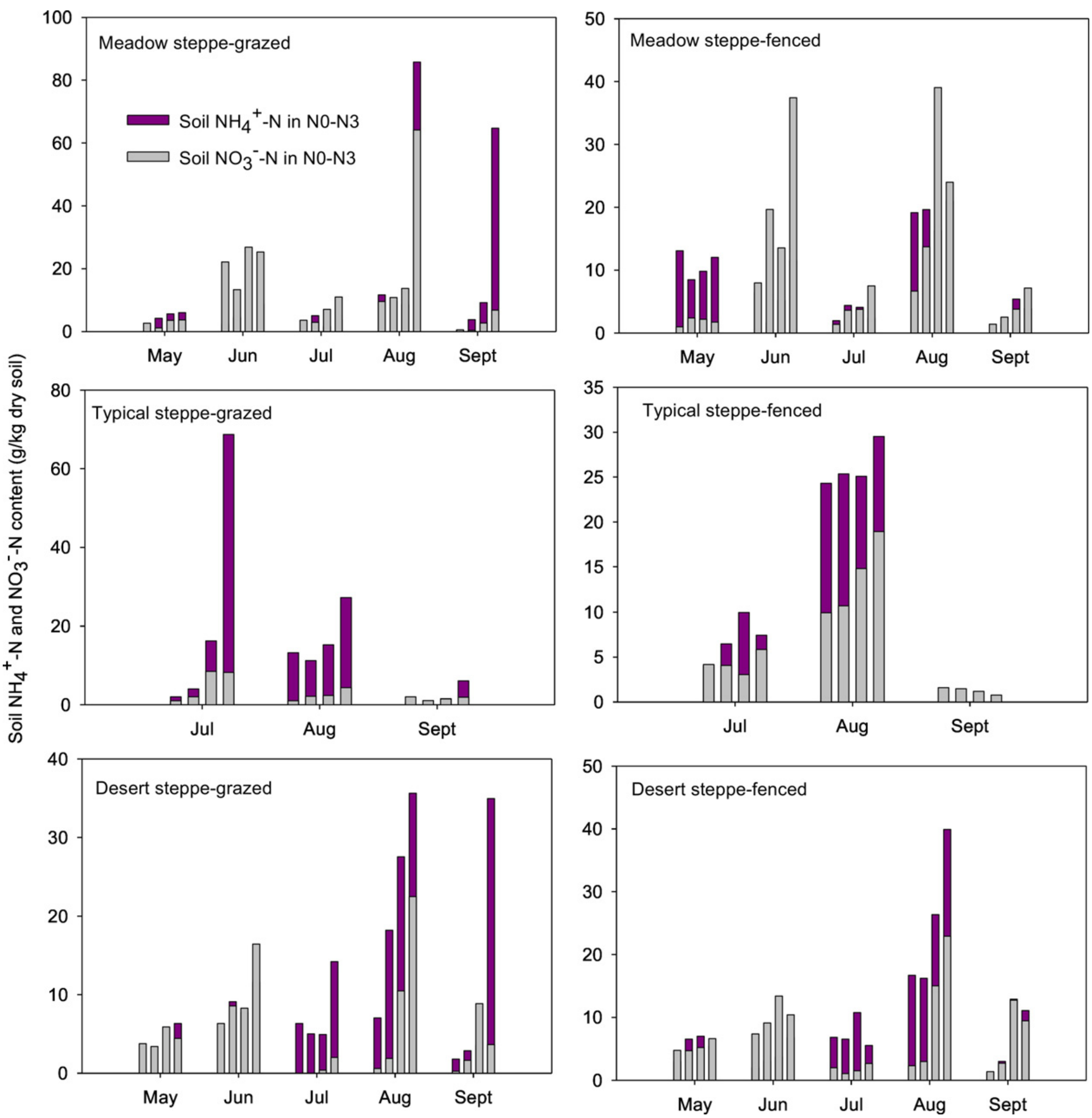

Fig. 4. Soil NH_4^+-N and NO_3^--N content in different treatments across various steppes during the growing season in 2012.

desert steppe in the western region in Inner Mongolia. Soil C and N content at different depths followed this order: meadow steppe > typical steppe > desert steppe, whereas the opposite trend for the averaged CH_4 uptake was found in these three different steppe types (Tables 1 and 2). Aronson and Helliker (2010) also reported that smaller amounts of soil N content tended to stimulate CH_4 uptake, whereas larger amounts tended to inhibit CH_4 uptake. The continental steppe, as a significantly different CH_4 sink, has been confirmed in many previous studies (Wang et al., 2005, 2009). Further investigation such as studying the influences of the types of added N fertilizer and N immobilization capacity in different steppe types is required.

Although some studies reported a positive correlation between CH_4 uptake and soil temperature (Wang et al., 2005), which is consistent with our findings, many other studies have found this relationship to be less significant in some regions (Holst et al., 2008). The controls on gas diffusivity, such as soil texture, bulk density, and soil moisture attained great importance in these regions (Holst et al., 2008; Smith et al., 2003). The differences in the Q_{10} values for the three steppe types indicated that the CH_4 fluxes in the typical steppe were much more sensitive to soil temperature than those in the meadow steppe and the desert steppe (Table 4). This further showed that the effect of soil temperature on the CH_4 flux depended on the type of steppe.

4.3. Effect of grazing management on CH_4 uptake

The magnitude of the cumulative CH_4 uptake (1.12–2.40 kg ha^{-1}) during the growing season in the grazed steppes was only 74–91% of that of the surrounding fenced steppes, with a range from 1.14 to 3.36 kg ha^{-1} (Table 2). This suggests that grazing exerts a considerable negative effect on CH_4 uptake in semi-arid steppes at regional scales. These findings also imply that reducing the grazing pressure on steppe would help increase the atmospheric CH_4 sinks in steppe soils, which was in agreement with the previous studies (Wang et al., 2009; Chen et al., 2011a, 2013; Liu et al., 2007). Wang et al. (2009) indicated that the CH_4 uptake in grazed grasslands was approximately 68% of that in ungrazed grasslands, with integrated CH_4 uptake rates ranging from 2.15 to 4.67 kg ha^{-1} in grazed grasslands and from 2.99 to 6.90 kg ha^{-1} in ungrazed grasslands. Chen et al. (2011a, 2013) reported that highly grazed steppe reduced the annual CH_4 uptake by 21–34%. Liu et al. (2007) reported that winter grazing significantly reduced the steppe CH_4 uptake during the growing season by 47%. However, some other previous studies have shown inconsistent effects of grazing management on CH_4 uptake (Wang et al., 2005; Saggar et al., 2007). In temperate semiarid steppes, Wang et al. (2005) did not detect significant differences in CH_4 uptake between grazed and ungrazed steppes. Tang et al. (2013) indicated that light grazing of steppe did not significantly change CH_4 uptake compared with un-grazed steppe, but moderate and heavy grazing reduced CH_4 uptake significantly by 6.8–37.9% ($P < 0.05$). The magnitude of the sink of CH_4 in steppe ecosystems might have changed significantly with the expansion of degraded grasslands (Zhao et al., 2017).

Grazing is a complex event in continental steppes (Wang et al., 2009; Zhu et al., 2015) since a range of factors such as fecal and urine deposition and changes in soil structure and aerobicity could change soil characteristics and affect CH_4 uptake. Three mechanisms may collectively or independently contribute to the significant reduction in CH_4 uptake in grazed steppes. Firstly, the reduction in soil CH_4 uptake most likely occurred because of a decrease in soil gas permeability. Animal trampling disturbs the topsoil and decreases the CH_4 diffusion and oxygen from the atmosphere into the soil profiles (Saggar et al., 2007; Liu et al., 2007; Zhu et al., 2015). Several parameters such as bulk density, soil moisture and litter layer characteristics may affect gas diffusivity and soil aeration (Smith et al., 2003; Holst et al., 2008; Liu et al., 2007). Overgrazing could lead to an increase in bulk density at the top soil surface (1.28 g cm^{-3}) compared to ungrazed sites (0.97–1.07 g cm^{-3}), indicating lower soil gas permeability (Liu et al., 2007; Chen et al., 2011a, 2013). Secondly, fresh animal excretion deposited on grazed sites were normally hotspot sources of CH_4. Although the most intensively grazed steppe experiences a net CH_4 uptake, the higher CH_4 emissions originating from the deposition of feces and urine in heavily grazed sites could offset the CH_4 uptake more than at other sites (Jiang et al., 2010; Chen et al., 2011a). Finally, grazing changes the community of soil methanotrophs compared to that in a typical steppe. Previous reports have suggested that the populations of two types of soil methanotrophs (Type I and Type II) were affected by the grazing intensity in Inner Mongolia (Zhou et al., 2008). Further studies need to be carried out to explore the mechanisms behind the relation between CH_4 uptake and grazing management in the experimental region.

4.4. Interaction of N deposition, steppe types, and grazing management

Across three different steppes, N deposition, steppe type, and grazing management was the main factors affecting CH_4 uptake (Table 3, $P = 0.000$), but their interactions were not significant (Table 3, $P = 0.931$). As simulated N deposition increased, soil CH_4 significantly decreased across the three different steppe types in this study (Fig. 3), but no significant interaction between N deposition and steppe types, between N deposition and grazing management was found in this study (Table 3, $P = 0.287$–0.772). Previous studies have shown that CH_4 uptake can be suppressed by N deposition to the soils in the temperate grasslands (Mosier et al., 1996), but that CH_4 uptake can also be promoted if sites are found to be strongly N-limited (Bodelier and Laanbroek, 2004). However, there was no effect of N deposition on CH_4 uptake across the different types of steppe (Phillips and Podrebarac, 2009). Jiang et al. (2010) reported that N deposition tended to reduce CH_4 uptake and that the differences caused by N deposition were all non-significant during the growing season in an alpine meadow. Li et al. (2012) showed significantly increased CH_4 uptake in response to N deposition during the growing season. Ambus and Robertson (2006) indicated that CH_4 uptake was overall unaffected by increasing N inputs in grassland communities during the growing season. Four years of increased N deposition did not significantly change the soil CH_4 sink in the degraded steppe in Inner Mongolia because the amount of added N was not enough to change the CH_4 uptake, and most of the supplied N was used for plant growth (Chen et al., 2013). These comparisons indicated that the effect of N deposition on CH_4 uptake was highly variable in different types of grassland ecosystems and that the observed differences might have been caused by differences in the climatic conditions and the types of vegetation and soil.

Repeated-measures of ANOVA indicated that grazing management significantly impact CH_4 uptake across meadow steppe, typical steppe, and desert steppe, respectively (Table 3, $P = 0.001$–0.170). Also, significant interaction between grazing management and steppe types was found in this study (Table 3, $P = 0.047$). Tang et al. (2013) indicated that light grazing would be the recommended practice since no significant change in CH_4 uptake was found; with a 6.8–37.9% reduction of CH_4 uptake, moderate grazing and high grazing ought to be discouraged during the growing season. In our study, the CH_4 uptake in plots with grazed steppe was detected based on practical stocking rates across various steppe types. The diversity of grazing practices in various steppes may limit the conclusiveness of studies of grazing effects on CH_4 uptake. Effect of different grazing intensity with the uniform stocking rates across different steppe types was of great importance in the interaction of grazing management and steppe types on CH_4 uptake in continental steppe.

4.5. Uncertainty analysis

Previous studies have generally focused on a single factor such as N deposition rate, grazing management, or steppe type on CH_4 fluxes in Inner Mongolia (Zhang et al., 2016, Zhang and Lu, 2017). However, there is little information available on how N deposition rates

interactively affect CH_4 uptake across different steppe types under grazed and fenced conditions in Inner Mongolia, China. Incomplete consideration of these impact factors across the entire steppe region in previous studies may lead to high uncertainty when the overall contribution of steppe ecosystems to the greenhouse warming effect is assessed. This study confirmed the individual and interactive impact of simulated N deposition, steppe types, and grazing management on CH_4 uptake in continental steppe in Inner Mongolia, China. A number of issues need careful attentions when interpreting the results obtained in this study.

Firstly, the low frequency of gas sampling and measurement might have caused biases. The low frequency (three times within one month) and the short measurement duration (only growing season from May to October in 2012) of CH_4 flux measurement would lead to bias in the estimation of averaged fluxes during the growing season, and also for the annual or seasonal accumulation of CH_4 uptake. It would also lead to uncertainty in quantification of the individual effect and interaction of N deposition, steppe types, and grazing management.

Secondly, residual effects or additive effects of different N application times in typical steppe on CH_4 uptake should be considered. Zhang and Lu (2017) reported the effects of the frequency and the rate of N enrichment on community structure in a temperate grassland, where changes in aboveground net primary productivity, plant species richness and shifts in dominant species were observed. This might further affect CH_4 uptake in continental steppe because of the variation of C and N in soil and plant, the soil characteristic and microbiology communities (Zhu et al., 2015; Zhang et al., 2016; Zhang and Lu, 2017). Further studies involving year-round, intensive measurement of CH_4 uptake and the same N application frequency and rate across different steppe types are needed.

Thirdly, we collected gas samples using the 100-ml polypropylene syringes with three-way nylon stopcocks and then transferred to a vacuum air trap (Tedlar, Delin Gas Package Co. Ltd., Dalian, China). Although we took the gas samples back to the lab for analysis within one week, the results of CH_4 uptake might be affected by the quality of the air trap. When interpreting the interaction of N deposition, steppe type, and grazing management on CH_4 fluxes in continental steppe, we should consider the uncertainties and biases generated during the analysis process of gas samples.

5. Conclusions

We investigated the interactive effects of simulated N deposition rates (0, 50, 100, and 200 kg ha^{-1}), steppe types (meadow steppe, typical steppe, and desert steppe), and grazing management (grazed and fenced) on CH_4 uptake during the growing season in Inner Mongolia, China. The three types of steppe played large roles as CH_4 sinks, which were significantly affected by the N deposition rate, the grazing management, and the steppe type. The magnitude of CH_4 uptake in the desert steppe was found to be significantly higher than that in the typical steppe and the meadow steppe. The fenced steppe significantly increased the CH_4 uptake when compared to the grazed steppe, suggesting that overgrazing may exert a considerable negative effect on CH_4 uptake. The soil CH_4 sink in the continental steppe decreased as the N deposition rate increased, supplying important proof of the feedback of different grassland ecosystems to global change. The N deposition rate is increasing, and it is reasonable to assume that elevated N deposition induced by anthropogenic reactive N emissions will most likely reduce CH_4 uptake in Inner Mongolia, China.

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